a3 Cont Molecular weight: about 43,000 (SDS-PAGE).

REMARKS

The Present Invention and Pending Claims

Claims 24-42 are currently pending and directed to a creatine amidinohydrolase (claims 24-37), a method for producing creatine amidinohydrolase (claim 38), a reagent for determination of creatine in a sample (claims 39 and 41), and a method for determining creatine in a sample (claims 40 and 42).

Amendments to the Claims

Claims 24, 25, 33, 35, 36, and 37 have been amended to point out more particularly and claim more distinctly the subject matter of the invention. In particular, claims 24, 25, 33, 35, 36, and 37 have been amended to recite that the creatine amidinohydrolase is encoded by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequences encoding the amino acid sequence of SEQ ID NO:1. This recitation is supported by the specification at, for example, column 4, lines 5-15. Claims 33 and 35-37 have been amended to recite the pH range and temperature at the optimal temperature and optimal pH, respectively, as supported by the specification at, for example, column 6, lines 47-49, and column 7, lines 12-19. No new matter has been added by way of these amendments. Separate documents setting forth the precise changes to the claims, as well as the text of all pending claims, are enclosed herewith.

The Office Action of February 12, 2002

The Office has rejected claims 24-42 under 35 U.S.C. § 112, first paragraph, as containing subject matter that allegedly was not described in the specification in such a way as to reasonably convey that the inventors had possession of the claimed invention at the time the patent application was filed, and for alleged lack of enablement. The Office has rejected claims 24-42 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Claims 24 and 28 have been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Japanese Patent Application Number 62-099182. The Office has declared the reissue oath/declaration to be defective. Reconsideration of these rejections is hereby requested.

Discussion of the Rejection under 35 U.S.C. § 112, first paragraph

The Office has rejected claims 24-42 under Section 112, first paragraph, as containing subject matter that allegedly was not described in the specification in such a way as to

reasonable convey that the inventors had possession of the claimed invention at the time the patent application was filed.

The Office contends that the specification is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus, because the properties of the creatine amidinohydrolase of the claims are ranges and only one property is given as a point number (molecular weight) (Office Action, pages 4-5). The Office asserts that the isoelectric point is an important characteristic that is omitted from the currently pending claims (Office Action, page 5).

The Office has similarly rejected claims 24-42 under Section 112, first paragraph, because the specification allegedly does not enable a person skilled in the art to make all the embodiments encompassed by the pending claims. Specifically, the Office contends that the specification does not support the broad scope of the claims which encompass any creatine amidinohydrolase having any structure and obtained from any source (Office Action, page 6).

As discussed above, the pending claims have been amended to recite a creatine amidinohydrolase encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1. The pending claims, as amended, properly define the subject matter of the invention as described and are enabled by the specification.

The omission of the isoelectric point from the pending claims does *not* cause those claims, especially as amended, to define subject matter not supported or enabled by the specification. The reasons for this view are set forth below.

The isoelectric point (pI) value of 3.5, as recited in the original claims, is a typographical error, and therefore the value was removed during prosecution of the reissue application. The pI value refers to the pH of a solution at which the total charges of an amphoteric electrolyte becomes 0 (i.e., neutral). The twenty different amino acids, which make up proteins, are amphoteric electrolytes having positive charges (e.g., amino group) or negative charges (e.g., carboxyl group). Each amino acid has a specific pI value. Accordingly, a protein composed of amino acids is an amphoteric electrolyte having a pI value unambiguously determinable from the amino acid sequence of the protein.

If the amino acid sequence of a protein has been determined, the pI value of the protein can be determined. Conversely, if the pI value of a certain protein is known, only general properties of an amino acid composition (e.g., the amount of acidic amino acids, and the amount of basic amino acids) can be inferred from the pI value. It is impossible to unequivocally derive the amino acid sequence of a protein from its pI value.

The pI value recited in the issued claims of the underlying patent was directly determined from test measurements. The measurements from such tests inevitably result in a

range of values (± a few percent), even when the test operation is completed appropriately with no mistakes. This means that the pI value in the issued claims of the underlying patent inherently included a certain variation (even putting aside the fact that the actual numerical value is a typographical error), as is true with any other pI value.

The amended claims recite a creatine amidinohydrolase encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1. Given the total number of the amino acids of the creatine amidinohydrolase that represents the source material recited in the pending claims (i.e., 404 amino acids), the difference in the amino acid sequence between the source material and mutants, or between different mutants, is expected to be 2-3% at most, so as to preserve the inherent function and recited physiochemical properties of the enzyme. This level of difference in the sequence is considered to make very little difference in the pI value.

This is illustrated, for example, by the pI values for a wild-type creatine amidinohydrolase derived from *Alcaligenes faecalis*, and mutants thereof. The following pI values were calculated by the use of Compute pI/MW tool at http:///www.expasy.ch/tools/pi_tool.html:

(a) wild-type creatine amidinohydrolase derived from *Alcaligenes faecalis* has a pI of 5.36;

(b) a mutant corresponding to pCRH273M1 (column 9, lines 34-39), wherein the 135th Arg has been substituted by Ala, has a pI of 5.29;

(c) a mutant corresponding to pCRH273M2 (column 9, lines 34-39), wherein the 135th Arg has been substituted by Ala and the 15th Glu has been substituted by Gly, has a pI of 5.35; and

(d) a mutant corresponding to pCRH273M3 (column 9, lines 34-39), wherein the 135th Arg has been substituted by Ala and the 104th Arg has been substituted by His, has a pI of 5.28.

Accordingly, the difference in the amino acid sequences of the source material and mutant creatine amidinohydrolases of the pending claims would not result in much difference in the pI value. The pending claims, as amended, inherently define creatine amidinohydrolases of a relatively narrow pI value range, rendering the omission of the pI value from the amended claims of no consequence regarding the requirements of Section 112.

For the foregoing reasons, the rejections under Section 112, first paragraph, should be withdrawn.

Discussion of the Rejection under 35 U.S.C. § 112, second paragraph

The Office has rejected claims 24-42 under Section 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention.

The Office contends that the K_m range is defined by the reaction of creatine in a coupling assay, but the reaction does not necessarily define pH, temperature, or the buffer under which the K_m range is measured (Office Action, page 7). The Km value is described in the specification at column 5, lines 16-26, as the value relative to creatine in a coupling assay using a sarcosine oxidase and a peroxidase. The method for determining the activity of the creatine amidinohydrolase is described at column 6, line 62, through, column 7, line 40, of the specification. With the description provided in the specification, one of ordinary skill in the art would be able to measure the K_m value, and therefore the K_m range recited in the pending claims is not indefinite.

The Office contends that the term "pH stability" is unclear because the amount of activity required to be stable is not defined (Office Action, page 7). The pH stability of a certain enzyme is determined from the residual activity after incubating the enzyme under certain conditions for a predetermined period. The term "pH stability" and the way to determine pH stability are known in the art, as shown by references AG-BA, submitted herewith in a supplemental IDS. The enzyme activity declines rapidly beyond a certain pH (see, e.g., Figure 10 of Chiba et al., *Agr. Biol. Chem. 37(8),* 1823-1829 (1973) (reference AK)), and those of ordinary skill in the art understand that the pH stability value represents the pH value just before the decline of the activity of the enzyme. Thus, the term "pH stability" is a common term that is understood in the art, and is not unclear as used in the pending claims.

The Office contends that the terms "heat stability" and "not more than about 50 °C" are confusing, because the activity of the enzyme is not defined and the range is open-ended (Office Action, page 8). The heat stability of a certain enzyme is determined from the residual activity after incubating the enzyme under given conditions for a predetermined period. The term "heat stability" and the means to determine heat stability are understood in the art, as shown by references AG-BA. For example, the residual activity of an enzyme is maintained at 100% to a certain temperature, beyond which the stability declines rapidly (see, e.g., Figure 11 of Chiba et al., *Agr. Biol. Chem. 37(8),* 1823-1829 (1973) (reference AK)). One of ordinary skill in the art understands that the "heat stability" temperature represents the temperature just before the rapid decline in activity at higher temperatures.

Moreover, the accepted method to express heat stability is by the upper limit of enzyme stability as in the pending claims. For example, in Figure 10 of Horikoshi et al., *Agr.*

Biol. Chem., 36(2), 285-293 (1972) (reference AG), no data is provided for temperatures lower than 40 °C because 100% residual enzyme activity at 40 °C implies stability in the temperature range of 40 °C and below. As an exceptional case, Kawai et al., *Agr. Biol. Chem., 35(11),* 1660-1667 (1971) (reference AM) sets 0 °C as a lower limit of heat stability; however, 0 °C is merely the lower limit of testing of the heat stability and does not mean that the enzyme lost activity below 0 °C. In fact, diagnostic assay reagents containing enzymes are typically stored at cold to room temperatures. Those of ordinary skill in the art consider that this temperature range, and particularly a refrigerating temperature range (about 0 °C to about 4° C), permits the most stable preservation of enzymes, since the possibility of inactivation becomes higher as the temperature rises.

Accordingly, the terms "heat stability" and "not more than about 50 °C" are not confusing to one of ordinary skill in the art, who would understand that the residual activity of the enzyme rapidly declines at temperatures above about 50 °C.

For the foregoing reasons, the pending claims particularly point out and distinctly claim the subject matter of the invention, and the rejection under Section 112, second paragraph, should be withdrawn.

Discussion of the Rejection under 35 U.S.C. § 102(b)

The Office has rejected claims 24 and 28 under Section 102(b) as allegedly anticipated by Japanese Patent Application Number 62-099182. The pending claims, as amended, recite a creatine amidinohydrolase encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1. JP 62-099182 does not disclose a creatine amidinohydrolase with this characteristic. In that JP 62-099182 does not teach or suggest every element of claim 24 or 28, JP 62 099182 does not anticipate claim 24 or 28, and the Section 102 rejection should be withdrawn.

Discussion of the Defective Oath/Declaration

The Office has declared the reissue oath/declaration to be defective, because the oath/declaration fails to contain a statement that all errors which are being corrected in the reissue application arose without any deceptive intention on the part of the applicant. A new reissue oath/declaration has been submitted with this response to Office Action to address this issue.

Conclusion

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Date: August 12, 2002

Certificate of Mailing Under 37 CFR 1.10		
"Express Mail" Label Number: EV065947155US		
Date of Deposit: August 12, 2002		
I hereby certify that this "Response to Office Action" and all accompanying documents are being deposited with the United States Postal Service "Express Mail Post Office To Addressee" Service under 37 CFR 1.10 on the date indicated below and is addressed to: Commissioner for Patents, Washington, D.C. 20231.		
Rick D. Madsen	Rick D. Madsen	August 12, 2002
Name of Person Signing	Signature	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Sogabe et al.

Art Unit: 1652

Application No. 09/940,941

Examiner: E. Slobodyansky

Filed: August 28, 2001

For: CREATINE AMIDINOHYDROLASE, PRODUCTION THEREOF AND USE THEREOF

AMENDMENTS TO CLAIMS MADE IN RESPONSE TO OFFICE ACTION DATED FEBRUARY 12, 2002

24. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

25. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

33. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

35. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

36. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

37. (Amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Sogabe et al.

Art Unit: 1652

Application No. 09/940,941

Examiner: E. Slobodyansky

Filed: August 28, 2001

For: CREATINE AMIDINOHYDROLASE, PRODUCTION THEREOF AND USE THEREOF

PENDING CLAIMS AFTER AMENDMENTS MADE IN RESPONSE TO OFFICE ACTION DATED FEBRUARY 12, 2002

24. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

25. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action:catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

26. The creatine amidinohydrolase of claim 24, which is stable at pH 5-8 (40 °C, 18 h preservation).

27. The creatine amidinohydrolase of claim 24, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

28. The creatine amidinohydrolase of claim 24, which has a molecular weight of about 43,000 (SDS-PAGE).

29. The creatine amidinohydrolase of claim 25, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

30. The creatine amidinohydrolase of claim 25, which has a molecular weight of about 43,000 (SDS-PAGE).

31. The creatine amidinohydrolase of claim 26, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

32. The creatine amidinohydrolase of claim 26, which has a molecular weight of about 43,000 (SDS-PAGE).

33. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

34. The creatine amidinohydrolase of claim 33, which has the following physicochemical properties:

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Heat stability: not more than about 50 °C (pH 7.5, 30 min).

35. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

36. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C (at a pH of about 6-8)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

37. A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutation of (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

~~Optimum temperature: about 40-50 °C (at a pH of about 6-8)~~

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE).

38. A method for producing the creatine amidinohydrolase of claim 24, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 24, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. A reagent for determination of creatinine in a sample, comprising a creatinine amidinohydrolase, the creatine amidinohydrolase of claim 24, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.